

# A DESCRIPTION OF THE COMPLETE METAMORPHOSIS OF THE SEA URCHIN *LYTECHINUS VARIEGATUS* CULTURED IN SYNTHETIC SEA WATER<sup>1, 2</sup>

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## ABSTRACT

The urchin *Lytechinus variegatus* was cultured through metamorphosis, from eggs and sperm collected from mature forms, in synthetic sea water, in the laboratory. A unicellular green alga was used as the nutritional source. A description and photographic record are presented of the complete development from fertilized egg to adult.

The general pattern of the metamorphosis of sea urchins has been described in some detail by Hyman (1955), who used larval forms of *Psammechinus*, *Paracentrotus*, *Echinus*, and *Anthocidaris* as examples. A photographic study of the larval development of *Arbacia punctulata* under laboratory conditions was published by Harvey (1956), who noted the difficulty of obtaining stages later than those of the newly metamorphosed adult. Tennent (1910), in a study primarily concerned with abnormality in development, reported the culture of *Lytechinus variegatus* in the laboratory. He did not describe the later metamorphic stages. A paper by Hinegardner (1969) described the development of *Lytechinus pictus* under laboratory conditions and provided information on culture techniques.

The present study describes the culture and development of *Lytechinus variegatus* in synthetic sea water and includes a photographic record of twenty-three stages from fertilization to adult.

## MATERIALS AND METHODS

Specimens of *Lytechinus variegatus* were obtained from Florida (The Gulf Specimen Company, Panacea, Florida) and kept at 21°C in ten-gallon aquaria containing synthetic sea water (Instant Ocean, Aquarium Systems, Inc., Wickliffe, Ohio). Specific gravity was maintained at 1.023 (salinity 32‰/100). The aquaria were fitted with undergravel filters covered with a three-inch layer of limestone as a filtering medium. The urchins were maintained from four to six months on a diet of frozen shrimp.

The fertile period of *Lytechinus variegatus* is from March to October. Gametes were obtained by injecting 5 ml of 0.5 M KCL into the peristomial membrane with a hypodermic needle. Few urchins survived for more than one to two weeks after injection (Tyler, 1966). Hinegardner (1969) reported that urchins injected with 0.1–0.2 molar acetylcholine-sea water solution are more likely to survive. Sperm were collected by pipette and stored without water in sterile plastic petri dishes. Eggs were collected by inverting the urchin over a beaker containing synthetic sea water. Both eggs and sperm were immediately refrigerated at 5°C to preserve their viability.

Sperm suspensions consisted of one to two drops of the concentrated sperm in 10 ml of synthetic sea water. Egg suspensions were prepared in 150-ml Erlenmeyer flasks containing 100 ml of synthetic sea water; a sufficient number of eggs was transferred by pipette from the stock container to form a thin layer of eggs on the bottom of the flasks. Both suspensions were kept in ice baths during their use in the laboratory; sperm suspensions were especially susceptible to deterioration, and the percentage of fertilizations decreased after 15–20 minutes. Egg

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suspensions and undiluted sperm kept at 5°C remained viable for an average of 24 hours. On one occasion fertilizations were obtained throughout a period of 40 hours. The number of abnormally developed embryos increased with the age of the gametes at the time of fertilization.

Fertilization was accomplished by combining two drops of sperm suspension and two drops of egg suspension on a depression slide and mixing quickly with a toothpick. Each slide was microscopically examined to confirm the formation of the fertilization membranes. The fertilized eggs from two slides prepared by this method were immediately transferred to a 125-ml glass jar containing 110 ml of synthetic sea water. The injection of one urchin of each sex provided sufficient eggs and sperm to establish 75 of the 125-ml cultures, each having approximately 100 fertilized eggs. Fertilizations were prepared one or more times at hourly intervals for a period of 24 hours in order to establish a continuing series of developing embryos. The jars were provided with loosely fitted plastic lids and evaporation was minimal, with no significant change in specific gravity occurring during the developmental period. The cultures were maintained at 23°C and were not aerated.

After 24 hours at 23°C, the embryos developed to the pluteus stage with a digestive tract. At this point the availability of an algal suspension to the free-swimming pluteus larvae was a critical factor. It was important that the algae be suspended; an initial loss of larvae was observed in cultures where the algae had settled to the bottom or the sides of the containers.

A freshwater unicellular green alga (12  $\mu$ ) resembling *Chlorella* was used as a nutritional source. Algal cultures were established several weeks prior to each series of fertilizations by adding a suspension of mixed freshwater algae from an established ten-gallon aquarium in the laboratory to an aerated one-gallon jar of synthetic sea water to which eight grams of soluble fertilizer had been added (HYPONeX 7-6-19, Hydroponic Chemical Company, Inc., Copley, Ohio). It is significant that a sufficient amount of freshwater algae survived and reproduced each time to maintain a stock marine culture of the suspended *Chlorella*-like green alga (which has not yet been identified).

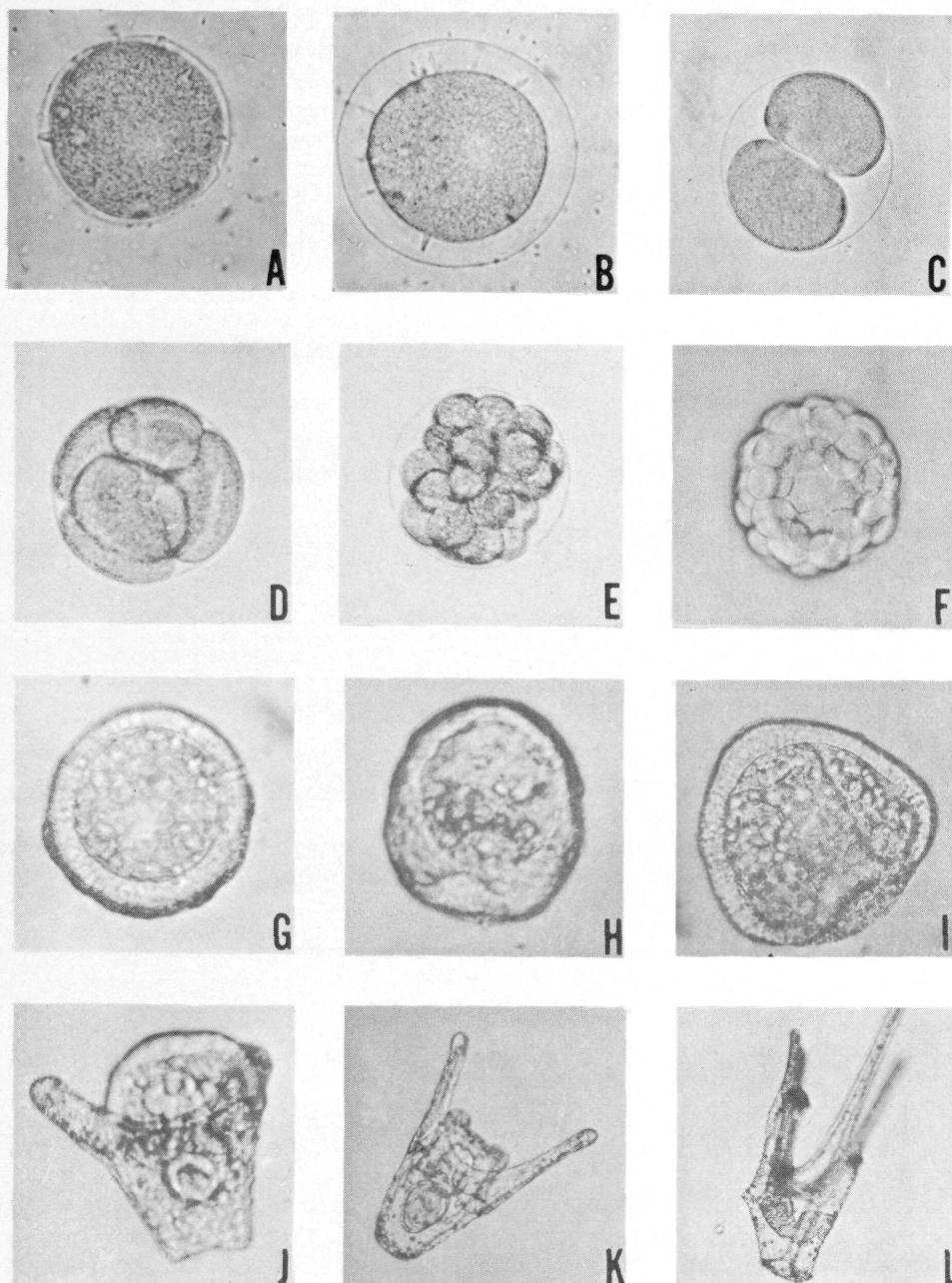
Five milliliters of this algal suspension were added to each culture container. The jars were placed six inches from a 40-watt cool-white fluorescent lamp fixture, which was positioned on the counter surface in such a way as to illuminate the cultures from the side. The light intensity at six inches was 280 footcandles, and the photoperiod was 12 hours.

For purposes of examination, developing embryos and larvae were removed from the containers by pipette and transferred to a drop of synthetic sea water on a depression slide. All photographs were taken of living specimens from slides prepared in the above manner. Coverslips were not used because of their tendency to distort or injure the larvae, especially while attempting to return them to the culture containers.

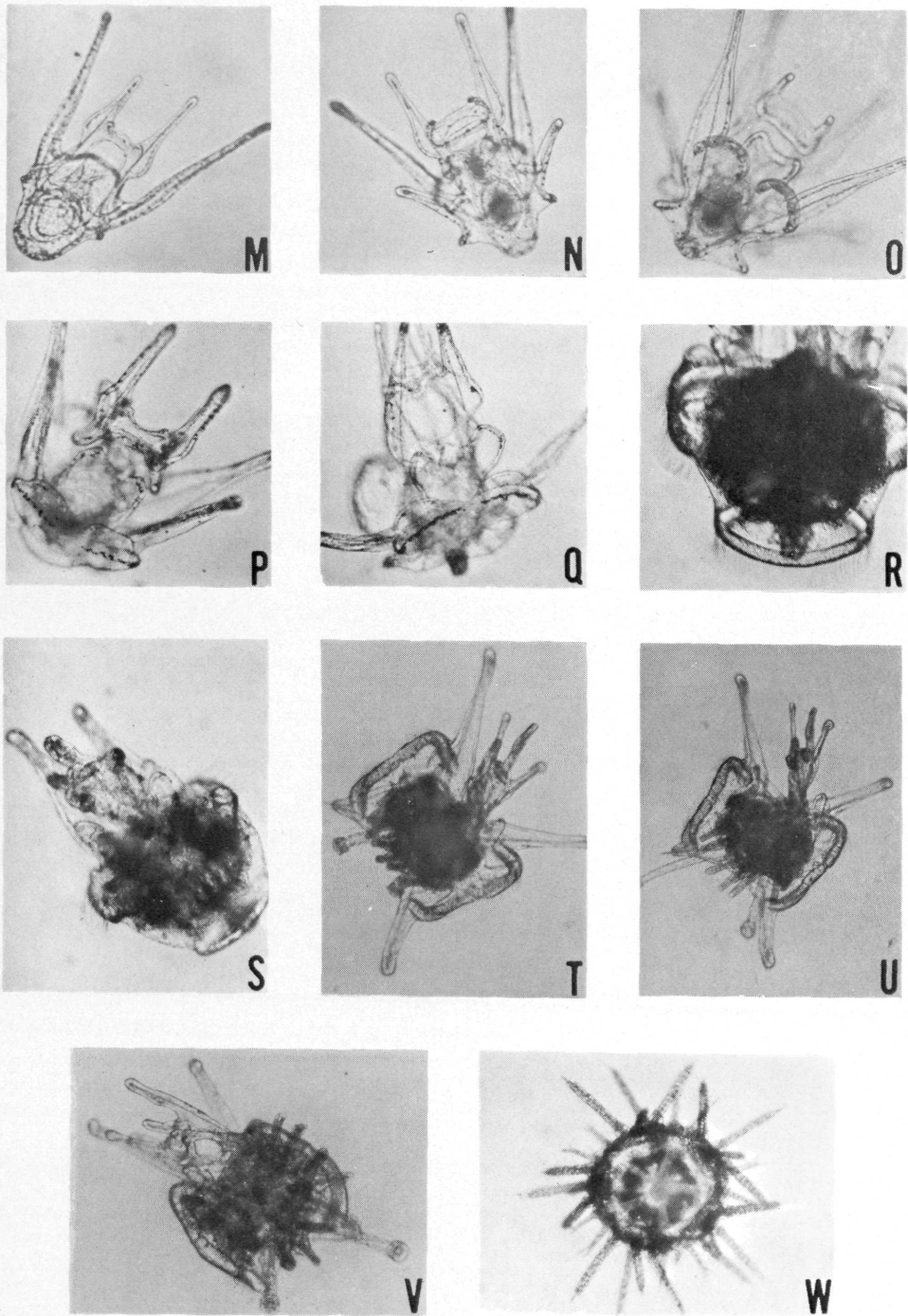
#### RESULTS

Four attempts to culture larvae through metamorphosis to adults were made during a 16-month period extending from March, 1967, to July, 1968. In the first trial, a few larvae reached advanced stages, but none metamorphosed to the adult stage. However, in the three subsequent trials, an average of 24 adult urchins was obtained. In each trial, the majority of the larvae progressed to a stage comparable to photograph N, Figure 1 (11 days at 23°C). At this point the number of surviving larvae steadily decreased. When maintained at a constant 23°C, the shortest developmental period was 33 days, as compared to 43 days under temperatures which fluctuated between 18° and 29°C.

A photographic record of the complete development of *Lytechinus variegatus* is shown in Figure 1. The photographs represent a combination of those from



Stages in metamorphosis of sea urchin *Lytechinus variegatus*.



Stages in metamorphosis of sea urchin *Lytechinus variegatus*.

three separate investigations. They are arranged in developmental sequence and are chronological, with the exception of photographs R and S. The investigation from which R and S were obtained was carried out under fluctuating temperatures, and all stages of development took longer to complete.

The following descriptions refer to Figure 1.

- A. 1 minute  $227 \times 0.11$  mm (actual size of organism)  
Fertilized egg showing fertilization membrane beginning to form.
- B. 3 minutes  $200 \times 0.11$  mm  
Fertilized egg with fertilization membrane complete
- C. 45-60 minutes  $218 \times 0.11$  mm  
First cleavage
- D. 75 minutes  $192 \times 0.13$  mm  
Third cleavage
- E.  $2\frac{1}{2}$  hours  $164 \times 0.14$  mm  
Morulla inclosed in fertilization membrane
- F. 4 hours  $164 \times 0.14$  mm  
Early blastula
- G. 6 hours  $170 \times 0.17$  mm  
Late blastula with cilia partially visible on perimeter
- H. 13 hours  $188 \times 0.16$  mm  
Early-ciliated gastrula with pronounced cells in interior which are the start of the mesoderm. At this stage, normal gastrulae were observed to move near the surface of the water, while those displaying abnormalities tended to remain at the bottom of the culture container.
- I. 18 hours  $188 \times 0.16$  mm  
Late gastrula. Although not evident in the photograph, echinochrome pigment cells were first observed in this stage.
- J.  $27\frac{1}{2}$  hours  $117 \times 0.30$  mm  
Pluteus larva having complete digestive tract and capable of feeding on unicellular algae. The well-defined opening in the lower half of the photograph is the anus. The postoral arms are beginning to form. The tips of the arms and the arched oral lobe behind them represent the leading front of the swimming embryo and form a funnel which directs algae into the mouth, which is under the oral lobe.
- K. 48 hours  $64 \times 0.47$  mm  
Further elongation of postoral arms. A pair of anterolateral arms are beginning to form from the knobbed rim of the oral lobe. The green algae in the mid-portion of the digestive tract are strikingly evident in the transparent larva.
- L. 3 days  $48 \times 0.83$  mm  
Side view of pluteus larva, showing normal orientation in the water and constriction of algae-filled digestive tract into three sections. The middle, or stomach, section and intestine, terminating in an anus at the right of the photograph, are clearly shown. The mouth and esophagus are partially obscured by the shorter pair of arms.
- M. 3 days  $48 \times 0.90$  mm  
Pluteus larva with postoral and anterolateral arms supported by well-formed skeletal rods. These rods developed from triradiate spicules which first become evident in the late gastrula (18 hours). The digestive tract runs centrally through the larva. The mouth, the clear opening at the base of the shorter (anterolateral) arms, is followed by a constricted, muscular esophagus, which exhibits peristalsis during feeding.
- N. 11 days  $50 \times 0.80$  mm  
Posterodorsal arms, the third pair to form, first appear. The darkened

- appearance of the bulbous stomach section is due to the concentration of algae.
- O. 16 days 46× 0.83 mm  
Development of arched pigmented ciliated bands between the postoral arms. Immediately above the bands is the mouth cavity, inclosed on the lower surface by a small concave edged lobe and on the upper surface by a larger overhanging fold, from which protrude two small preoral arms. These preoral arms are the fourth and last pair to form. Characteristic thorns of the skeletal rods are distinguishable in the postoral arms.
- P. 22 days 52× 0.83 mm  
Stage exhibiting numerous concentrations of dark pigment cells, which contain the bright red echinochrome pigment. The migration and coalescence of these pigment cells is fully described in Young (1958).
- Q. 27 days 43× 0.93 mm  
Stage showing tendency of postoral and anterolateral arms to be drawn together, while posterodorsal arms remain extended. A pedicellaria at the base of the larvae is located centrally between the two ciliated bands. The larvae appear less active and spend considerable time on the bottom of the culture container.
- R. 37 days  
In this enlargement of the basal portion of the larva, the pigmented arches appear to form a ciliated ring which encircles the pedicellaria. Increased differentiation of adult tissue accounts for the dense appearance of the interior of the larva. Photographs R and S are not in chronological sequence because they were obtained from the investigation carried out under fluctuating temperatures in which these stages of development took longer to complete. Under a constant temperature of 23°C, these stages are reached at approximately 30 days.
- S. 37 days 50× 0.90 mm  
Commencement of degeneration of larval arms. Activity of adult spines and tube feet is evident in the interior of the larva at this stage.
- T. 33 days 20× 2.1 mm  
A continued degeneration of larval tissue accompanied by the emergence of the adult form, as demonstrated by the appearance of adult spines and tube feet. A protruding tube foot may be seen slightly below left center of the larva.
- U. 33 days 20× 2.1 mm  
A series of five well-formed spines can be seen extending from the left-hand side of the adult mass.
- V. 36 days 20× 2.1 mm  
Final stage before completion of metamorphosis. Numerous well-formed spines and extended tube feet are evident. Larval structures are discarded or absorbed at this point.
- W. 37 days 52× 0.80 mm  
Ventral view of a newly metamorphosed adult.

#### CONCLUDING REMARKS

Nutrition is the most important factor in the culture of the larvae, and the standardization of a method for culturing suspended unicellular algae in synthetic sea water should greatly increase the percentage of larvae which reach the adult stage. Crowding is another factor which may be responsible for a decrease in the number of surviving larvae during the later stages of development. During the course of this study, larvae were transferred from jars which seemed particularly crowded, but no concerted effort was made to investigate this factor. Aeration was attempted during an earlier trial, but this had a disrupting effect on the larvae and caused increased evaporation from the container.

*Lytechinus variegatus* is becoming increasingly popular as an experimental organism in embryological and cytological studies. An important reason for this is the fact that it can be maintained at room temperature. The fact that this urchin can be cultured to an adult stage under laboratory conditions is of interest to those engaged in such research, for it is possible to observe the effects of experimental procedures carried out during earlier stages of development. The primary significance of this study is that it indicates that, because of the simplicity of the culture method and of the required materials, it is possible to include investigation of the complete metamorphosis of this organism in developmental courses at the graduate and undergraduate level. Sea-urchin studies in most of these courses have previously been concluded with the appearance of the pluteus larva.

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